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Absence of Maternal Behavior in Rats With Lithium/Pilocarpine Seizure-Induced Brain Damage: Support of MacLean's Triune Brain Theory

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PEREDERY, O., M. A. PERSINGER, C. BLOMME AND G. PARKER. *Absence of maternal behavior in rats with lithium/pilocarpine seizure-induced brain damage: Support of MacLean's triune brain theory.* PHYSIOL BEHAV **52**(4) 665–671, 1992.— Female rats, with and without maternal experience, received limbic seizure-inducing (SC) injections of lithium and pilocarpine. Following the subsequent parturitions, these rats displayed a complete absence of maternal behavior. Rats that did not display seizures after receiving the lithium/pilocarpine injections displayed behaviors that were comparable to normal controls. Although the multifocal limbic, thalamic, and cingulate damage abolished maternal care, there was no evidence of aberrant effects upon fecundity, litter size, or mammary function; infanticide was negligible. The pattern of brain damage involves the evolutionarily more recent thalamocingulate system of mammals and supports MacLean's theory that these pathways are required for normal mother–offspring interaction.

Maternal bonding Seizure Lithium Pilocarpine Rats Parturition Brain damage Thalamus Entorhinal Cingulate MacLean

PAUL MacLean (9,10) has argued that three forms of behavior distinguish the evolutionary transition from reptiles to mammals:

1. nursing, in conjunction with maternal care,
2. audiovocal communication for maintaining maternal–offspring contact, and
3. play.

These behaviors are hypothesized to have emerged with the phylogenetic development of the thalamocingulate division of the brain. This division, which is not well developed in reptiles, primarily involves the anterior cingulate and the co-innervations from the mediodorsal, anterior medial, and intralaminar nuclei of the thalamus.

Experimental ablations of structures within the mesiobasal limbic system (amygdala–pyriform cortices) and the thalamocingulate division have strongly implicated the latter structures. Slotnick and his colleagues (17,18) reported that discrete lesions in the interpeduncular nucleus, amygdala, or overlaying piriform cortex did not adversely affect maternal behavior in rodents. Anterior cingulate and septal lesions seriously disrupted this behavior, resulting in mortality in some offspring. Whereas moderate disruptions in general maternal behavior have been shown following discrete lesions within the preoptic area of the hypothalamus (5,6), disruptions in the initiation but not the maintenance of maternal behavior have been reliable sequelae to destruction of the paraventricular nucleus of the hypothalamus (5).

The primary limitation of all of these studies is the specificity of the lesion. If maternal behavior, as proposed by MacLean, requires the integrity of the thalamocingulate system, then its entire disruption would be required before a strong test of the hypothesis, i.e., abolishment of maternal behavior, would occur. The concept would be analogous to that reported by Cohen (1) for conditioned cardiac acceleration in pigeons; as long as any one of the three pathways that mediate this response remained intact, the behavior was displayed.

Several researchers (3,15,16) have found that a systemic injection of 3 mEq/kg of lithium followed 24 h later by 30 mg/kg of pilocarpine induces maintained epileptogenicity in the rat. The subsequent insidious brain damage occurs throughout large regions of the diencephalon and telencephalon (2,13–16). However, maximum neuronal necrosis and reactive gliosis occurs within areas that include all of MacLean's thalamocingulate system and its cognate mesiobasal limbic structures, specifically the hippocampus, amygdala, and overlaying cortices; using Paxinos

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and Watson's (12) terminology, they are the cingulate cortex, piriform cortex, endopiriform nucleus, most amygdaloid nuclei, primarily CA1 and CA2 of the hippocampal formation, the anteriomedial thalamic nucleus, the mediodorsal thalamic complex, the ventromedial thalamic nucleus, the suprageniculate nucleus, and the paratenial nucleus. These areas are major relays that maintain the integrity of the mammalian limbic system.

We hypothesized that the devastating, multifocal damage to the limbic system, evoked by the lithium/pilocarpine seizures, would be a specific test of MacLean's theory that these regions are essential for family-related behaviors. We hypothesized that the strategic positioning of the necrosis should effectively eliminate maternal behavior without altering the physiological responses, such as mammary preparation, or eliciting aberrant hypothalamically mediated changes, such as eating the young.

EXPERIMENT 1

METHOD

Animals

In two separate blocks of experiments (separated by 2 months) a total of 24, 80- to 100-day-old female Wistar albino rats, obtained from Charles River Suppliers (Hull, Quebec), served as subjects. They were maintained in standard racks (three per cage) within the usual environment: 12/12 hour light/dark cycle and room temperature (20°C).

Procedure

A total of 16 of the rats received 3 mEq/kg of lithium chloride (subcutaneously) followed 4 h later by 30 mg/kg of pilocarpine (also 1 cc/kg; SC). Half the numbers of these rats displayed forepaw clonus and rearing within 1 h after the pilocarpine injection while the other eight females did not display any overt seizures [this discrepancy in seizure occurrence within female rats is typical (16)]. One h after the pilocarpine injections, all 24 rats received 25 mg/kg (subcutaneously; 10 mg/cc) of acepromazine (Atravet, Ayerst Laboratories, Montreal) in order to minimize the risk of mortality.

One month later, females were housed (two to three per cage) with males for 2 weeks. A total of 21 females (seven controls, seven rats that did not have seizures with lithium pilocarpine and 7 rats that had seizures) became pregnant. Four days before expected parturition, each rat was housed singly in 45 × 25 × 20 cm plastic cages that contained one-quarter inch corncob (Bed-o-cob). Beginning on the day of parturition, each rat was observed for 10 min, three times per day (1000, 1400, and 1800 h) during the light cycle from days 1–4 postpartum. With each observation each mother was scored on the following measures: body-spreading (over pups), integrity of the litter (all rats retrieved and in nest), and the nest form. The scores ranged from 0 (no crouching; all pups spread throughout the cage; no arrangement of the corncob in a corner of the cage, respectively) to 2 (normal crouch; all pups within a group; normal nest, respectively).

During one of the observation periods, nests (when present) were disturbed and three pups from each litter were examined to insure the presence of stomach milk. Four days after parturition, the mothers were decapitated. A complete postmortem was completed for all major abdominal and thoracic organs; close examination of the mammary glands was completed. The brains were removed and fixed in EFA for 48 h and then processed for paraffin embedding (14). Ten-μm coronal sections were obtained every 200 μm between the caudal mesencephalon and rostral telencephalon. Each section was stained with toluidine blue and examined under a light microscope at 100× and 400×. Every structure, as defined by Paxinos and Watson (12), was evaluated to determine necrosis (neuronal cell dropout) and gliosis.

Because the data were qualitatively distinct, most of the comparisons between the females that had received the lithium/pilocarpine and either had seizures or not, and the controls, involved chi-square analyses. Other quantitative comparisons utilized one-way analyses of variance (ANOVA). All analyses utilized SPSSX software on a VAX 4000 computer.

RESULTS

The means and standard deviations (in parentheses) for the numbers of pups born for the control, nonseizure control and seizure groups were 12.1 (1.8), 13.6 (2.7), and 11.4 (1.3), respectively; a one-way ANOVA indicated no statistically significant, $F(2, 18) = 2.97$, $p > 0.05$, group differences.

Within 1 h of parturition all of the control and nonseizure control mothers were engaging in normal maternal activities such as sequestering the pups and anal licking. However, none of the seven seizure mothers displayed these behaviors, $\chi^2 = 21.00$, $p < 0.001$. As shown in Fig. 1, these females delivered, removed some of the amnions, but then totally ignored their offspring (often stepping on them); although there was no evidence of infanticide, one or two pups per litter of the mothers that had seizures were partially consumed; the damage appeared more related to gnawing by the mother rather than to cannibalism.

The scores for squatting, maintaining the group, and nest formation during postnatal days 1–4 for the three groups were qualitatively distinct. Whereas group scores for these three measures for the drug control and control mothers all ranged between 1.9 and 2.0 (all standard deviations less than 0.1), the scores for the mothers that had seizures never obtained a score above 0; the females totally ignored their pups, despite blatant separation cries, and often walked on them. Only one of these mothers displayed a limbic seizure (forepaw clonus, rearing, and falling) during the observation periods. Milk was always evident within the stomachs of the pups within the two reference groups. Because the pups of the mothers that had seizures were scattered over the corncob bedding, each one could be examined; there was never evidence of stomach milk.

The seizure mothers were killed at the end of postpartum day 4. Postmortems performed on these seven mothers revealed developed and lactogenic mammary glands; all abdominal and thoracic organs were qualitatively healthy. Brains from the control group and from the group that had received the lithium/pilocarpine but had not had seizures revealed no pathology. However, the brains of the mothers that had seizures were characterized by conspicuous neuronal dropout, necrosis, and gliosis at 100× to 400× magnification. Figure 2 is a composite of the brain damage that was evident within the mothers that displayed the absence of maternal behavior. Primary necrosis (approximately 70 days after lithium/pilocarpine; 25 days after impregnation) was evident within the entorhinal–piriform region (with invasion into the underlying amygdala), dorsal thalamus, and portions of the cortices, including the cingulate region.

Whereas the entorhinal region showed massive cell loss, the thalamic lesions were dominated by deep staining of cell fragments and gliosis. Pervasive damage was particularly evident within the dorsomedial complex, the suprageniculate nucleus, the posterior nucleus, and the anteriomedial nucleus. Neuronal dropout and gliosis characterized the remaining areas of brain damage, especially within the anterior cingulate region. The pattern of damage was similar to what we have observed within the

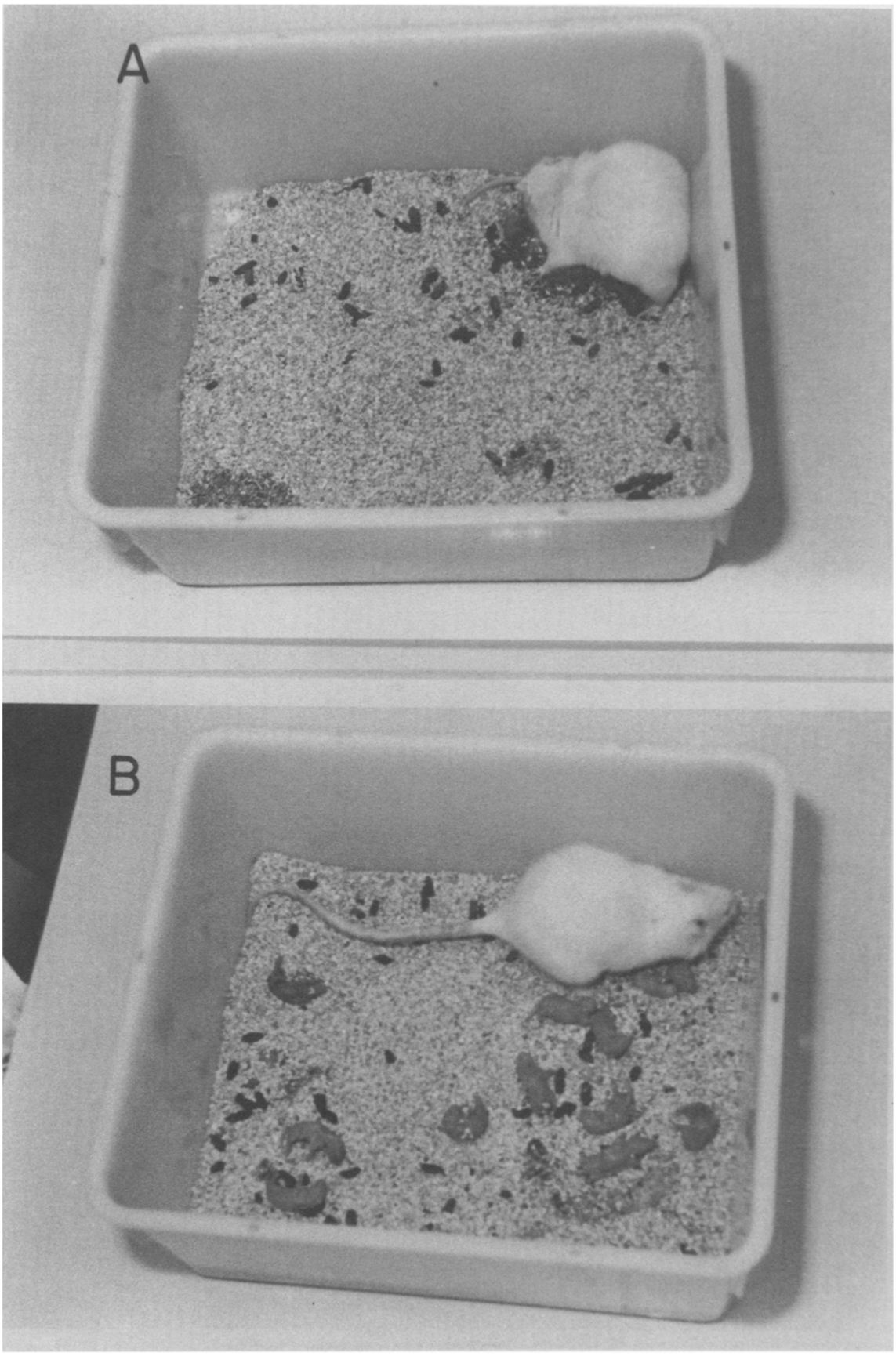

FIG. 1. (A) A normal female rat displaying maternal behavior. (B) Aberrant maternal behavior displayed by a female rat that had been seizured with a single injection of lithium and pilocarpine 2 months previously.

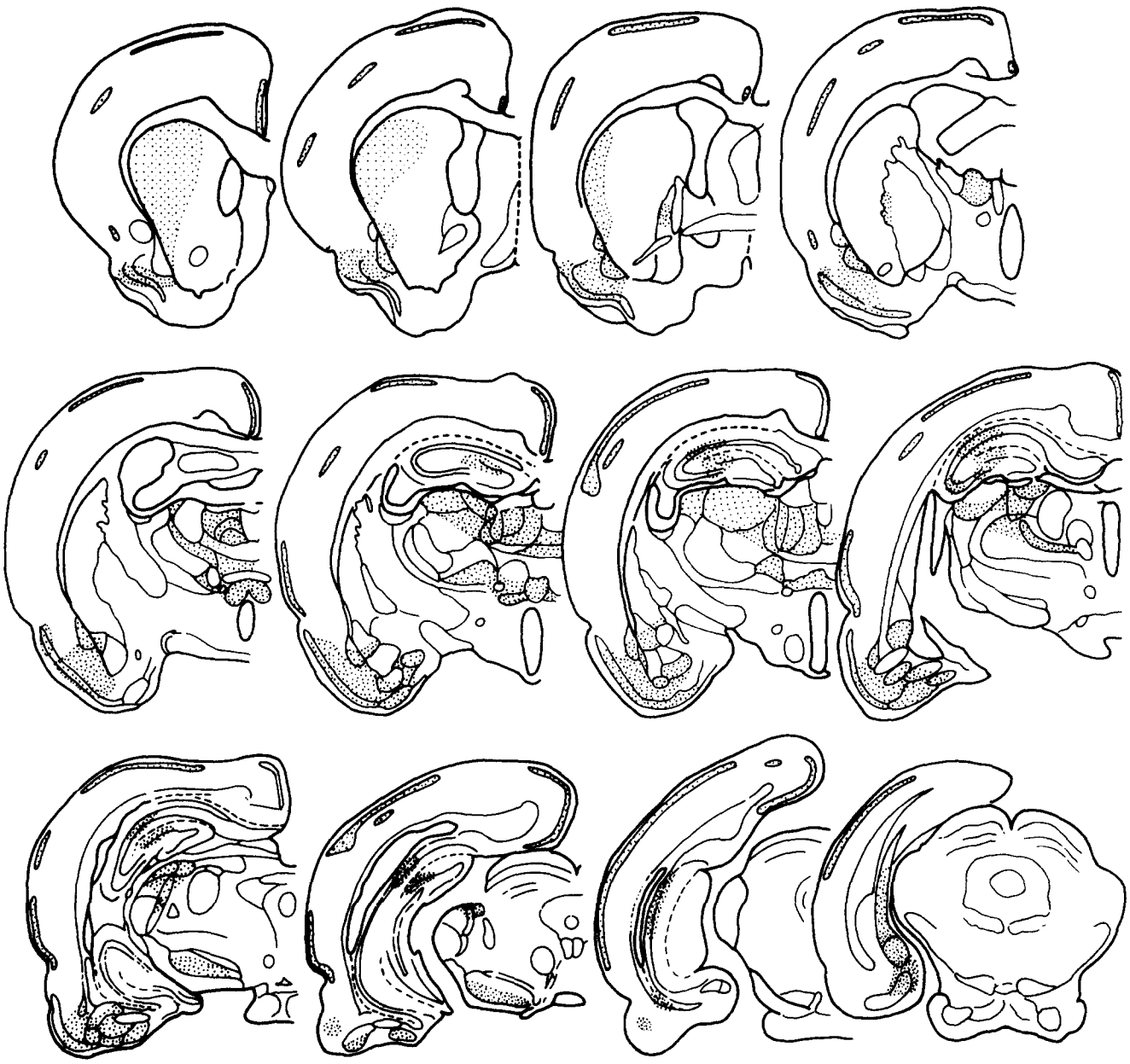

FIG. 2. Distributions of necrosis within the brains of the females that had seizured following the systemic lithium/pilocarpine injection. There were three major types of cytoarchitectural damage: the entorhinal region displayed cystic lesions while the thalamic areas displayed intensified staining of neuronal fragments; the remaining areas showed conspicuous gliosis.

brains of male rats after a comparable postseizure duration (2,13,15,16).

EXPERIMENT 2

METHOD

Subjects

A total of 36 female albino Wistar rats, approximately 180 days of age, served as subjects.

Procedure

Approximately 30 days after the weaning of the first litters, 18 primiparous mothers and 18 nulliparous (virgin) rats were randomly assigned to one of two groups. One group (nine primiparous; nine nulliparous) received (SC) 3 mEq/kg of lithium chloride followed 24 h later by 30 mg/kg of pilocarpine (SC); within 30 min after the onset of the seizure they received 30 mg/kg of acepromazine. The other 18 rats (nine primiparous; nine nulliparous) were not given injections of lithium or pilocarpine but were administered the acepromazine.

About 3 weeks later all of the females (two to three per cage) were exposed continuously to Wistar males that were similar in age. The females were observed once every 2 days in order to discern approximate parturition. About 2 to 3 days before parturition, the rats were removed and placed into the single plastic cages that were described in Experiment 1. Numbers of offspring

were recorded. The evaluations described previously were employed to measure maternal behavior. Four days after parturition the mothers in which seizures had been induced were decapitated. The mammary glands were examined.

Brains of seven of the mothers (three multiparous; four primiparous) were removed, fixed, and processed (14). Coronal sections, 10 μm in thickness, were stained with toluidine blue for every 150 μm of brain space between the midbrain and the anterior commissure. Possible statistically significant differences in damage scores, based upon an ordinal ranking system (2) for clusters of functionally relevant nuclei (2), between multiparous and primiparous brains were examined by ANOVA. Because the range of the scores was between 0 and 7, we concluded that any conspicuous differences should be evident even with this small sample size.

In addition, the presence or absence of damage was determined according to a nominal rating system (13) for each of the structures, as defined by Paxinos and Watson (12) within this volume of brain. In order to compare the distribution and magnitude of seizure-induced brain damage with our primary (male) data base, histopathological scores from 7, randomly selected age-matched males were obtained (13). Chi-square analyses were completed between the brain damage scores for the female brains and male brains. All analyses were completed by SPSSX software.

RESULTS

The numbers of rats that became pregnant were: controls primiparous, 7/9; seizure primiparous, 7/9; controls multiparous, 8/9; and seizure multiparous, 6/9. Two-way ANOVA as a function of parturitional history and seizure history demonstrated no significant differences in litter sizes for the seizure-nonseizure, $F(1, 24) = 0.50$, $p > 0.05$, or primiparous-multiparous, $F(1, 24) = 1.75$, $p > 0.05$, groups; the interaction was also not statistically significant. The grand mean and standard deviation were 12.5 and 4.5, respectively.

Not one of the primiparous or multiparous females in which seizures had been induced displayed any form of maternal behavior (mean score = 0). All of the primiparous and multiparous control mothers displayed appropriate maternal behavior (means between 1.8 and 2.0, SDs between 0.1 and 0.2). Three of the mothers in the seizure groups ate their litters between 12 h and 72 h postpartum; unlike the other 10 mothers in these groups, these three females displayed no evidence of milk in the mammary region.

There were no statistically significant differences [all $F(1, 5) < 2.00$] in the severity of damage within major structures of the diencephalon, subcortical telencephalon, or cortices between multiparous or primiparous mothers in which the seizures had been induced. Comparison of the percentage of presence or absence of discernable damage within the brains of the seven females that were evaluated for histopathology and the seven age-matched male brains indicated comparable proportions of damage for most of the 75 structures that were examined. This means that, like males in which these seizures were induced, no discernable damage was found within any hypothalamic nuclei while damage was invariably found within structures such as the supragenicular nucleus (13,15).

Structures in which there was a significant discrepancy in incidence of damage are shown in Table 1. Females displayed significantly more incidence of damage within the insular cortices, parietal cortices, frontal cortices, temporal cortices, cingulate cortices, lateral septum, posterior nucleus of the thalamus, and nucleus of the lateral olfactory tract, compared to the males.

TABLE 1

STRUCTURES FOR WHICH THE PERCENTAGES OF MALE AND FEMALE (MOTHER) BRAINS DISPLAYING CONSPICUOUS DAMAGE (AT 400× LIGHT MICROSCOPY) WERE STATISTICALLY SIGNIFICANT USING PAXINOS AND WATSON'S ABBREVIATIONS

Structure	Males (%)	Females (%)
IC (prim. AI)	14	86*
Cg	0	57†
Fr (cortex)	0	57†
Par (cortex)	0	71*
Te (cortex)	0	71*
LS	0	57†
LOT	0	57†
Po	57	100‡
AHiA	0	43‡

* $p < 0.01$.
† $p < 0.05$.
‡ $0.05 < p < 0.10$.

DISCUSSION

These results indicate that this specific type of limbic seizure-induced, multifocal brain damage completely abolished maternal behavior without:

1. affecting fecundity,
2. altering litter size,
3. promoting infanticide, or
4. influencing mammary production.

Although multiple nuclei of the thalamus and limbic region were affected, as well as the limbic and neocortex, the structures of MacLean's thalamocingulate system were particularly devastated. The dramatic absence of maternal behavior in these rats supports Paul MacLean's concept (9,10) that the integrity of this system is essential for maternal bonding and audiovocal communication between mother and offspring.

The contribution of the cellular damage to the amygdala, hippocampus, or overlaying entorhinal cortices to maternal behavior, at least in primiparous mothers, is not likely. In male rats, comparable lesions, induced by the lithium/pilocarpine injections, promote aggression (15,16); there was no direct or indirect evidence of aggressive behavior displayed by these females. This conclusion would be commensurate with those of Slotnick and his colleagues (17,18) who also found no significant effect of amygdaloid lesions upon maternal behavior.

That the abolishment of maternal behavior was an artifact of other correlates of the multifocal brain damage is not likely. Even though male rats that had seizures with this procedure: 1) display severe disruption in spatial memory that is strongly correlated with the extent of damage within the dorsomedial nucleus (2) and 2) exhibit reduced conditioned taste aversion (20), there has always been residual capacity for these behaviors. The aberrant females in this study still consumed food and water, thus indicating that neither spatial memory or basic hypothalamic mechanisms had been disrupted. Although we did not measure prolactin directly, this latter supposition was certainly supported by the normal appearance of the mammary glands in these rats. The occurrence of conspicuous infanticide in the three mothers in which the mammary glands displayed an absence of milk suggests that this neuropeptide may inhibit this behavior.

Conspicuous factors, such as disruption by seizure activity, are not likely. During the 84 observation periods (seven rats, 3/

day; 4 days), only one overt seizure was ever observed. Although protracted covert seizures were likely, they are not likely to have been persistent. Subtle although clearly discriminable facial (protrusion and clouding of eyes), specific body posture, and oral automatisms are common correlates of electrical discharges that remain within the mesiobasal structures. These behaviors were never observed during the 4-day observation period. In addition, a maintained confusional state would have interfered with normal appetitive behavior for which there was no evidence.

The failure for these brain-damaged mothers to respond to the separation cry suggests that specific thalamic structures are involved with this behavior; some of these nuclei, traditionally at least, have relatively obscure functions. For example, the suprageniculate nuclei could be intimately involved with the neuropathways associated with the response to the separation cry and subsequent retrieval activity. This structure, as well as the adjacent lateral posterior nucleus, projects to the amygdala and has been implicated in the processing of the emotional significance of acoustic stimuli (7). The hyperresponsiveness of the defective mothers in this study to peripheral noises (e.g., movement of experimenter, hand clapping) yet complete lack of orientation towards their offspring suggests a specific rather than a generalized hearing deficit.

Maternal behavior is a multivariate behavior (11) that involves nest building, postpartum licking, retrieval, suckling, and the display of appropriate behavior by the pups. A recent unpublished study by Therrien (19) indicated that the offsprings of seizured mothers were normal; cross-fostered normal pups were ignored by these mothers while cross-fostered pups from the epileptic mothers were accepted by normal mothers. Pregnant females with a history of limbic seizures also failed to build (shredded paper) nests while normal females displayed this behavior. We conclude that the seizure-induced brain damage abolishes the entire domain of maternal behavior.

The specificity of the extent and pattern of the damage within the rat brain is conspicuous and deserves comment. Mesencephalic structures, except for the substantia nigra reticulata, are never affected [at least within the limits of light microscopy (13,15)], despite reciprocal connections with thalamolimbic regions. There is graded damage within the diencephalon, from the near-always devastated suprageniculate nucleus to the never affected hypothalamus. Within the telencephalon, the neostriatum shows minor diffuse damage (usually restricted to the face, oral, and forelimb regions), yet the ventral striatum, including the rich sources of cholinergic neurons, is intact.

Considering the mechanism by which lithium is presumed to affect the neuronal membrane (4), our results suggest a compositional similarity between the plasma membranes of the recent phylogenetic structures that mediate maternal behavior: this prediction would be a logical extrapolation of MacLean's hypothesis. Because the preadministration of lithium is assumed to preferentially hydrolyze an inositol phosphate of the cell membrane that enhances the vulnerability to the subsequent injection of subthreshold concentrations of a muscarinic agent, the specificity of the brain damage suggests a persistent commonality in the subtle membrane chemistry of structures that share phylogenetic emergence. The idea is not novel: genetic, system-specific proteins have also been demonstrated by the shared antibody-binding capacity for membranes of neurons that compose the traditional limbic system as well as nuclei not hereto included (8).

The major potential counterargument to our conclusions concerning the thalamocingulate system involves the incidence of damage within the cortices of the female rats compared to the male rats. The brains of the seven mothers that were examined in this study displayed a much greater incidence of damage within several cortices. If we assume that the magnitude (proportion) of incidence within a group of individual brains is often similar to the quantitative measures of distribution of damage within the individual brain (13), then female rats would display significant (microscopically conspicuous) damage within more than 50% of the cortices. As reported by Numan (11), Beach's original 1937 study showed that removal of 40% but not 20% of the cerebral cortices in rats completely abolished maternal behavior.

To our knowledge, researchers have utilized single, surgical lesions (5,11,17,18) or hormonal manipulations (6) to study maternal behavior; the major effects involved either fragmentation or reduction of maternal behavior. The lithium/pilocarpine-induced pattern of brain damage evokes multifocal lesions that involve all of these effective areas and hence abolishes maternal behavior. Other researchers have also demonstrated the importance of multifocal lesions in order to study complex behaviors. For example, Cohen (1) found that autonomic conditioning was reduced but still present when any one of three distinct tract systems was lesioned; the heart rate conditioning was totally abolished only when all three tracts were lesioned. Specific patterns of seizure-induced brain damage might be used in a similar manner to study behavior.

REFERENCES

1. Cohen, D. H. The neural pathways and informational flow mediating a conditioned autonomic response. In: DiCara, L. V., ed. Limbic and autonomic nervous system research. New York: Plenum; 1974: 223–275.
2. Harrigan, T.; Peredery, O.; Persinger, M. A. Radial maze learning deficits and mediodorsal thalamic damage in context of multifocal, seizure-induced brain damage. Behav. Neurosci. 105:482–486; 1991.
3. Honchar, M. P.; Olney, J. W.; Sherman, W. R. Systemic cholinergic agents induce seizures and brain damage in lithium treated rats. Science 220:323–325; 1983.
4. Honchar, M. P.; Vogler, G. P.; Gish, B. G.; Sherman, W. R. Evidence that phosphoinositide metabolism in the rat cerebral cortex stimulated by pilocarpine, physostigmine and pargyline in vivo is not changed by chronic lithium treatment. J. Neurochem. 55:1521–1525; 1990.
5. Insel, T. R.; Harbaugh, C. R. Lesions of the hypothalamic paraventricular nucleus disrupt the initiation of maternal behavior. Physiol. Behav. 45:1003–1041; 1989.
6. Koch, M.; Ehret, G. Estradiol and parental experiences but not prolactin are necessary for ultrasound recognition and pup-retrieving in the mouse. Physiol. Behav. 45:771–776; 1989.
7. LeDoux, J. E.; Farb, C.; Ruggiero, D. A. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J. Neurosci. 10:1043–1054; 1990.
8. Levitt, P. A monoclonal antibody to limbic system neurons. Science 223:299–301; 1984.
9. MacLean, P. D. Evolution of audiovocal communication as reflected by the therapsid-mammalian transition and limbic thalamocingulate division. In: Newman, J. D., ed. The physiological control of mammalian vocalization. New York: Plenum; 1988:185–207.
10. MacLean, P. D. The triune brain in evolution. New York: Plenum; 1990.
11. Numan, M. Maternal behavior. In: Knobil, E.; Neill, J. et al., eds. The physiology of reproduction. New York: Raven; 1988:1569–1645.
12. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. New York: Academic Press, 1986.

13. Peredery, O.; Persinger, M. A. Exploration of functional pathways through multivariate statistical analyses of quantitative patterns of seizure-induced brain damage in the rat (submitted).
14. Persinger, M. A. Handling factors not body marking influence thalamic mast cells numbers in the preweaned albino rat. Behav. Neural Biol. 30:448–459; 1980.
15. Persinger, M. A.; Bureau, Y.; Kostakos, M.; Peredery, O. Behaviors of rats with insidious brain damage induced by seizures following single peripheral injections of lithium and pilocarpine (submitted).
16. Persinger, M. A.; Makarec, K.; Bradley, J-C. Characteristics of limbic seizures evoked by peripheral injections of lithium and pilocarpine. Physiol. Behav. 44:27–37; 1988.
17. Slotnick, B. M. Disturbances of maternal behavior in the rat following lesions of the cingulate cortex. Behaviour 24:204–236; 1967.
18. Slotnick, B. M.; Nigrosh, B. J. Maternal behavior in mice with cingulate cortical, amygdala, or septal lesions. J. Comp. Physiol. Psychol. 88:118–127; 1975.
19. Therrien, S. Loss of nest building, maternal care and absence of maternal response in rats in which lithium-pilocarpine seizures had been induced. Unpublished Psychology thesis; 1992.
20. Venuogopal, M.; Persinger, M. A. Conditioned taste aversion in rats with a history of lithium/pilocarpine induced limbic seizures. Neurosci. Lett. 90:177–180; 1988.